Formaldehyde Council

Via Electronic Mail

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Re: Comments for Consideration by the NTP Board of Scientific Counselors on the Draft Substance Profile for Formaldehyde, 75 Fed. Reg. 21,003 (April 22, 2010)

Dear Dr. White:

I. Introduction and Summary

The Formaldehyde Council, Inc.[1] (FCI) appreciates the opportunity to provide comments to the National Toxicology Program's (NTP) Board of Scientific Counselors (BSC) concerning NTP's Draft Substance Profile for Formaldehyde, which BSC will peer review at its June 21-22, 2010 meeting.[2] The BSC is charged with determining whether the scientific information cited in the Draft Substance Profile is technically correct, clearly stated and supports NTP's policy decision concerning formaldehyde's listing status in the 12th Report on Carcinogens (RoC).[3]

The Formaldehyde Draft Substance Profile (Profile) states, "Formaldehyde is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in humans and supporting studies on mechanisms of carcinogenesis."[4] The Profile concludes that:

> Epidemiological studies have demonstrated a causal relationship between exposure to formaldehyde and cancer in humans. Causality is indicated by consistent findings of increased risks of nasopharyngeal cancer, sinonasal cancer, and myeloid leukemia

[1] FCI is a group of leading formaldehyde producers and users who are dedicated to promoting the responsible use and benefits of formaldehyde and ensuring its accurate scientific evaluation. FCI members in clued American Forest and Paper Association; Arclin; Atlantic Methanol Company; Celanese Corporation; Certain Teed Corporation; Cytec; DB Western, Inc.; Dow Chemical Company; DSM Melamine; E.I. du Pont de Nemours and Company; Formica Corporation; GEO Specialty Chemicals; Georgia-Pacific, LLC; Hexion Specialty Chemicals, Inc.; Kitchen Cabinet Manufacturers Association; Methanex Corporation; Methanol Holdings (Trinidad) Limited; National Funeral Directors Association; Owens Corning; Panolam Industries International; and Troy Corporation.

[2] *See* 75 Fed. Reg. 21,003 (April 22, 2010).

[3] *See* NTP Report on Carcinogens Review Process, available at http://ntp.niehs.nih.gov/?objectid=FA925F34-F1F6-975E-775C81773747D452 (last updated Sept. 8, 2009).

[4] NTP, *Draft Report on Carcinogens Substance Profile for Formaldehyde*, 1, available at http://ntp.niehs.nih.gov/NTP/RoC/twelfth/2010/DrftSubProfiles/Formaldehyde20100421.pdf.

among individuals with higher measures of exposure to formaldehyde (exposure level or duration), which cannot be explained by chance, bias, or confounding.

Based on a review of the science and the application of NTP's criteria:

- the human, animal and other data for nasopharyngeal cancer (NPC) do not provide sufficient evidence that supports listing formaldehyde as a *known to be a human carcinogen*, but may be interpreted to support a listing as *reasonably anticipated to be a human carcinogen*,
- the human, animal and other data for sinonasal cancer do not provide sufficient evidence that supports listing formaldehyde as either *known to be a human carcinogen* or *reasonably anticipated known to be a human carcinogen.*
- the human, animal and other data for myeloid leukemia do not provide sufficient evidence that supports listing formaldehyde as either *known to be a human carcinogen or reasonably anticipated known to be a human carcinogen.*

FCI submitted comments on February 11, 2010, in response to the Expert Panel's recommendation to list formaldehyde as a known human carcinogen. Those comments address the same three endpoints. FCI continues to support the analysis of the scientific data with regard to nasopharyngeal cancer and sinonasal cancer in our February 11, 2010 (pages 16-17 and 21 are attached for ease of reference), but address here only the interpretative issues raised with regard to myeloid leukemia.

- The chemistry and biochemistry of formaldehyde is well-known. The Profile conflicts with this literature in constructing an explanation for how exogenous inhaled formaldehyde, which does not change normal endogenous concentrations in the blood at any feasible exposure level, can nevertheless travel to distant sites and initiate the leukemogenic process there. In doing so, the Profile misinterprets the cited literature and also overlooks studies demonstrating the implausibility of formaldehyde causing myeloid leukemia at distal sites, such as Lu *et al.* (2010).
- Of the four studies that NTP identifies as the most informative for evaluating the risk of myeloid leukemia, only one reported a statistically significant exposure-response relationship. Thus, the weight of the evidence does not support a conclusion that exposure to formaldehyde is causally associative with myeloid leukemia.
- Using an observed versus expected mortality test to evaluate the carcinogenicity of formaldehyde, the three major human formaldehyde occupational cohort studies do not demonstrate an increased risk of myeloid leukemia due to formaldehyde exposure
- Although cited in the Profile, the meta-analysis conducted by Bachand *et al.* (2010) did not demonstrate a significantly elevated risk of myeloid leukemia due to formaldehyde exposure, even after taking into account the proportionate-mortality cohort studies reporting increased risks of myeloid leukemia. The Zhang *et al.* (2009) meta-analysis failed to minimize heterogeneity among data sets.
- A recent *in vivo* study by Meng *et al.* (2010), not included in the draft Profile, reports that formaldehyde-induced mutagenicity is unlikely to play a role in nasal tumorigenesis.

There is little, if any, credible evidence of formaldehyde-induced hematotoxicity in humans, even though such effects would be expected if formaldehyde was in fact a leukemogenic carcinogen, regardless of its proposed mode of action. The draft Profile describes hematological effects as being observed; however, the underlying data are not scientifically credible. NTP also relies upon the preliminary and controversial results of Zhang *et al.* (2010), which FCI believes raises more questions than provides answers.

II. Cancer Studies in Humans

A. Nasopharyngeal Cancer

[No further comment. *See* FCI 2/10 Comments at 16-17.]

B. Sinonasal Cancer

[No further comment. *See* FCI 2/10 Comments at 3, 21.]

C. Myeloid Leukemia

NTP asserts that epidemiological studies demonstrate a causal relationship between exposure to formaldehyde and myeloid leukemia in humans:[5]

> *The most informative studies for evaluation of the risk of myeloid leukemia are the large cohort studies of industrial workers (the NCI, NIOSH, and British cohorts) and the NCI nested case-control study of lymphohematopoietic cancer in embalmers. Three of these four studies found elevated risks of myeloid leukemia among individuals with high exposure to formaldehyde, as well as positive exposure-response relationships.*[6]

For a chemical to be listed as *Known To Be A Human Carcinogen* as NTP proposes for formaldehyde, it must meet the criterion that:

> There is ***sufficient evidence*** of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.[7]

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[5] *See* Profile at 1.

[6] *Id*. at 4-5.

[7] *See* Listing Criteria at http://ntp.niehs.nih.gov/index.cfm?objectid=03C9CE38-E5CD-EE56-D21B94351DBC8FC3. NTP classifies substances as Reasonably Anticipated To Be Human Carcinogen based on the following criteria:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded,

or

there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in

It is assumed for the following comments that for the important purpose of establishing a causal relationship between exposure to formaldehyde and myeloid leukemia that "*elevated risks*" and "*positive exposure-response relationships*" are those that had attained statistical significance as this term is typically interpreted. If this is not the case then it is incumbent that NTP describe and justify its reliance on other, non-standard criteria as the basis for its conclusions. This is a critical point since, for better or worse, statistical significance is the only way that chance findings can be objectively eliminated as an explanation for study results. As noted in the EPA (2005) cancer risk assessment guidelines, "*The general evaluation of the strength of the epidemiological evidence reflects consideration not only of the magnitude of reported effects estimates and their* ***statistical significance*** *but also of the precision of the effects estimates and the robustness of the effects associations*."

As a threshold matter, the Profile's proposed classification of formaldehyde as a *known human carcinogen* is based upon the characterization of studies as demonstrating "elevated risks" and "positive exposure-response relationships." However, these descriptors are used to describe data that do not meet the criteria for statistically significant causal relationships. In its *Guidelines for Carcinogen Risk Assessment*, the U.S. Environmental Protection Agency (EPA) affirmed the critical role of statistical significance in evaluating the strength of epidemiological evidence.[8] Further, the Information Quality Act (IQA) requires that analytic results "be developed using sound statistical . . . methods."[9]

As the BSC is well aware, when results are not statistically significant at some chosen level of statistical confidence, it denotes the absence of a pattern, relationship or difference that may be taken as evidence of a causal effect to the chosen degree of confidence. Here, the absence of statistical significance means the absence of a difference sufficient enough for a conclusion to be adopted as to the occurrence of a causal effect.[10] Non-statistically significant differences in data could well be due to chance, or could be statistical artifacts resulting from an inadequate sample size, etc. For purposes of determining the presence of "sufficient evidence," NTP should base its interpretation on statistically significant data.

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multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset,

or

there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

[8] *See* U.S. EPA Risk Assessment Forum, *Guidelines for Carcinogen Risk Assessment*, EPA/630/P-03/001F, 2-12 (March 2005) ("The general evaluation of the strength of the epidemiological evidence reflects consideration not only of the magnitude of reported effects estimates and their statistical significance, but also of the precision of the effects estimates and the robustness of the effects associations.").

[9] Pub. L. No. 106-554, § 515, 114 Stat. 2763A-153 to 2763A-154, 44 U.S.C. § 3516 note (2000).

[10] Strictly speaking, a statistically significant difference only justifies rejection of the null hypothesis; it does not demonstrate the correctness of any other hypothesis. But the concordance of several statistically significant findings, combined with other factors can rise to a level of sufficiency that justifies a judgment that some other hypothesis is indeed correct.

The epidemiological studies NTP references are those by Beane-Freeman *et al*. (2009), Hauptmann *et al*. (2009), Coggon *et al*. (2003) and Pinkerton *et al*. (2004). Here, we analyze each of these studies for statistical significance with respect to RRs or SMRs or exposure-response trends. In two of the three studies, chance findings could not be objectively eliminated as an explanation for the results. Additionally, we address NTP's treatment of the meta-analyses conducted on these studies.

1. Beane-Freeman *et al*. (2009)

Beane-Freeman *et al*. (2009) analyzed the National Cancer Institute's (NCI) cohort study on industrial workers exposed to formaldehyde, which is by far the largest and most informative study in its class. The NCI authors stated that the data for myeloid leukemia lacked statistical significance.

> *For the highest peak exposure, there was a **non-statistically significant** elevated relative risk for myeloid leukemia (RR = 1.78; 95% CI = 0.87 to 3.64, P trend = .13). When including all person-years (both unexposed and exposed), the P trend was .07. For the highest average intensity, there was a **statistically nonsignificant** increase for myeloid leukemia (RR = 1.61; 95% CI = 0.76 to 3.39; P trend = .43).*

Beane-Freeman et al. (2009) also found that "There was no evidence that risks increased with cumulative number of peaks > 4.0 ppm or for duration of exposure for any cause of death evaluated (data not shown)." Consequently, the NCI cohort study does not demonstrate a causal relationship between exposure to formaldehyde and myeloid leukemia, as NTP states in the Profile.

While the NCI study is clearly the largest of the cohort studies the findings pertaining to leukemia are substantially confounded by the indisputable statistically significant deficits in mortality from leukemia in the unexposed (SMR=0.38, 95% CI 0.10-0.97) and low exposed (SMR=0.50, 95% CI 0.28-0.81) used for comparisons (See previous 2/11/09 comments). These deficits unequivocally influence the trends reported in both the Hauptmann et al. (2003) and Beane-Freeman et al. (2009) studies. All of this (in addition to the discovery that more than 1000 deaths had been missed in the 2003 study that substantially altered the findings) was explained in comments submitted on Feb. 11, 2010. It is unknown why the critical re-analyses of the NCI data by Marsh et al. are ignored when they show quite convincingly that the reported findings by Hauptmann et al. (2003) and Beane-Freeman et al. (2009) are not as straightforward as they might appear.

Beane-Freeman *et al*. (2009) found no statistically significant data to support a causal relationship between formaldehyde exposure and myeloid leukemia. This study does not provide sufficient evidence on which to base a known-to-be-a-human-carcinogen classification.

2. Pinkerton *et al*. (2004)

Pinkerton *et al*. (2004) prepared an updated analysis of the National Institute for Occupational Safety and Health (NIOSH) cohort study of garment workers exposed to formaldehyde. Again, NTP's reliance on this study is misplaced because the study did not demonstrate a statistically significant exposure-response trend. The Profile states:

> In the NIOSH cohort study of garment workers, elevated risks of death from myeloid leukemia were found for all workers and for subgroups of workers with the highest exposure or longest latency. SMRs were highest among workers with longer exposure duration (≥ 10 years), longer latency (≥ 20 years), or earlier year of first exposure (before 1963, when exposure levels were higher).[11]

While the myeloid leukemia Standardized Mortality Ratios (SMRs) for workers exposed to formaldehyde for ≥ 10 years was elevated, it was not statistically significant. Similarly, while the SMRs for workers with longer latency (≥ 20 years) were significantly elevated, there was no significant trend (i.e., exposure-response) for this exposure. In addition, the SMR for myeloid leukemia in workers with both 10 or more years of exposure and 20 or more years since first exposure was not significantly elevated (SMR=2.43, 95% CI 0.98-5.01). NTP either overlooked or failed to mention that, when analyzed by year of first exposure (compared to U.S. referent rates), none of the myeloid leukemia SMRs for exposure prior to 1963, 1963-1970, or 1971 or later were significantly increased. Instead, the primary significant findings are those that were calculated using the procedure of multiple cause mortality, in which other potentially contributory causes of death were considered (e.g., SMR=2.55, 95% CI 1.1-5.03 for myeloid leukemia in workers with both 10 or more years of exposure and 20 or more years since first exposure). The basis for this type of analysis (Steenland et al. 1992) is rather explicit that this methodology is of less value for cancer, in which other contributory causes are less likely to be listed on death certificates.

Even the authors of this study concede that the results were "*not conclusive*" and supported "*a possible relation between formaldehyde exposure and myeloid leukemia mortality*." Thus, Pinkerton *et al*. (2004) does not provide sufficient evidence on which to base a known-to-be-a-human-carcinogen classification.

3. Coggon *et al*. (2003)

Coggon *et al*. (2003) presents an extended follow-up of a large cohort of British chemical workers exposed to formaldehyde. This study is particularly noteworthy because it likely involved the highest exposures to formaldehyde of the four key epidemiological studies upon which NTP relies in assessing the relationship between formaldehyde exposure and myeloid leukemia in humans.

NTP acknowledges that in this cohort, "*no increased risk of leukemia was found for formaldehyde exposure."* [12] Indeed, the study showed no significant increase of any form of leukemia in the total cohort (SMR = 0.91, 95% CI 0.50-2.07) or in a sub-cohort of men with the highest exposure to formaldehyde (SMR = 0.71, 95% CI 0.31-1.39). Yet, despite this assessment, NTP unjustifiably attempts to diminish the import of Coggon *et al*.'s (2003) findings by stating, "*[T]his study did not evaluate myeloid leukemia specifically, and exposure-response analyses were limited; exposure was assessed as 'high' or 'ever,' and the assessment was not calendar-year-specific*."[13] Clearly, however Coggon *et al*. (2003) does not provide sufficient

[11] Profile at 5.

[12] Profile at 5.

[13] *Id*.

evidence on which to base a known-to-be-a-human-carcinogen classification and weighs against such a classification.

4. Hauptmann *et al*. (2009)

Hauptmann *et al.* (2009) demonstrated a statistically significant trend between exposure (years of working as embalmer) and myeloid leukemia, but, these findings, as is true of most studies involving embalmers, pathologists, and anatomists, are attributed to either reporting bias, some exposure other than formaldehyde-related substances in the embalming fluid, or to infectious agents.[14] In the recent embalmer study of Hauptmann *et al.* (2009), formaldehyde exposure is never measured but rather is inferred from the number of embalmings.

Embalming fluids are complex mixtures including numerous other chemicals along with formaldehyde. The mixture of chemicals in embalming fluids has changed over the years. Because the number of embalmings was one of the best predictors of risk of leukemia according to Hauptmann *et al.* (2009), it could be that another component of embalming fluids is related to the increased risk.

Due to the short time that this study has been available, it is possible that further analyses will reveal additional questions, but the following are some of the primary concerns with Hauptmann *et al.* (2009) (K. Mundt, personal communication).

- The study evaluates deaths occurring between 1960 and 1986 as reported on death certificates, but as late as 1992, NCI (SEER) did not report study results on cancer types being evaluated because SEER questioned the validity of these diagnoses as reported on death certificates.
- Important differences are apparent between myeloid (acute and chronic combined) leukemia cases and the control group and several time-dependent co-factors appear not to have been adequately considered or controlled in the analyses. This is a critical factor because myeloid leukemia is also a disease of aging.
- Standard statistical analyses were unreliable due to the fact that there was only one unexposed myeloid leukemia case.
- Myeloid leukemia cases and the controls had nearly identical mean estimated values for 8-hour time weighted average and peak formaldehyde exposure, which is inconsistent with the authors' interpretations.

While Hauptmann *et al.* (2009) may provide suggestive evidence of a relationship between formaldehyde exposure and myeloid leukemia, limits to the study design and statistical analyses lead to the conclusion that this study does not provide sufficient evidence on which to base a known-to-be-a-human-carcinogen classification and weighs against such a classification. Even accepting the results of this study on face value, it is the only one of the four studies upon which the conclusion that formaldehyde is causally related to myeloid leukemia is based that supporting this conclusion.

5. Summary and Meta-Analyses

[14] *See* Harrington and Shannon (1975), Walrath and Fraumeni (1983, 1984), Stroup et al. (1986), Hayes et al. (1990).

The above analysis clarifies that when the four most important epidemiological studies are evaluated under the rule of statistical significance, only the data from one study can be reasonably interpreted to support a causal relationship between formaldehyde exposure and myeloid leukemia in humans. This contrasts with the draft Profile's description of three of the four studies supporting such a conclusion. Notwithstanding the methodological problems inherent in the one study that observed a statistically significant trend (Hauptmann *et al.* (2009), the weight of evidence does not support a conclusion that exposure to formaldehyde is causally associative with myeloid leukemia.

Another common sense way to consider this body of data is to employ the approach taken by the Agency for Toxic Substances and Disease Registry (ATSDR 2000) in reviewing the carcinogenicity of polychlorinated biphenyls (PCBs). There, ASTDR simply compared the observed and expected mortality from all studies and used a statistical test to determine if there was a significant difference. When this approach is applied to the three major human formaldehyde cohorts that allow its utilization (Beane-Freeman *et al.* (2009), Pinkerton *et al.* (2004), and Coggon *et al.* (2003)), a total of 152 leukemia cases were observed while 153.2 would be expected. Simply put, there is nothing in this extensive body of data on more than 50,000 occupationally-exposed individuals suggesting an increased risk of myeloid leukemia.

Comparison of Observed and Expected Leukemia Mortality in Formaldehyde-Exposed Workers

Cohort	#Workers	Observed	Expected
Beane-Freeman	25,000	116	≈ 116
Coggon	14,000	12	13.2
Pinkerton	11,000	24	≈ 24
Total	50,000	152	153.2

As noted by NTP, several meta-analyses have been undertaken on the Beane-Freeman *et al.* (2009), Pinkerton *et al.* (2004), and Coggon *et al.* (2003) studies.[15] FCI, however, disagrees with the statement in the draft Profile that the meta-analysis conducted by Zhang *et al*. (2009) is the most informative, given its documented methodological flaws. *See* Feb. 11, 2010, FCI Comments at 8.

It is generally recognized that a meta-analysis of a disparate body of data may likely be inherently flawed due to the problem of heterogeneity. While Zhang *et al.* (2009) reported a statistically significant relative risk (RR), the authors used a questionable approach for selecting and combining studies. Rather than relying on the metrics of "ever" or "never" as used by Collins and Lineker (2004) and Bachand *et al*. (2010) to minimize subjective judgments and heterogeneity, Zhang *et al*. (2009) used different, far more subjective measures of exposure. The authors selected only one method of exposure from each study even when several were examined, resulting in reliance upon peak exposure for some studies, average exposure for others, cumulative exposure for still others, and exposure duration for the balance. Moreover, if several categories or levels of exposure were examined, they took data from only the highest among them, and what constituted a "high" category also varied considerably among studies, depending on how each study established gradations of exposure. As a consequence, the

[15] Profile at 6.

comparisons across studies are very heterogeneous, and it is not clear whether a comparable question was being examined in each case, which can lead to unreliable results in a meta-analysis. Thus, the result reported by Zhang *et al.* (2009) is not the most informative of the available meta-analyses. This is confirmed by its lack of concordance with the meta-analyses of Collins and Lineker (2004) and Bachand *et al*. (2010), which both stratify and analyze the data based on their separate consideration of low-exposure and high-exposure industries and use of consistent measures of exposure.

The Profile also addresses the meta-analysis conducted by Bachand *et al*. (2009), stating (emphasis added):

> A meta-analysis by Bachand et al. (2010) **did not** find a significantly elevated risk of myeloid leukemia (summary RR = 1.09, 95% CI = 0.84 to 1.40). However, this analysis did not include the proportionate-mortality cohort studies (studies that compared the proportions of deaths between the study population and a reference population), which reported increased risks of myeloid leukemia."[16]

In taking this position, NTP made a demonstrably incorrect assumption. As noted by Bachand *et al.* (2010)(emphasis added):

> Relevant leukemia and NPC case-control studies with reported odds ratios (ORs), cohort studies with reported relative risks (RRs) or standardized mortality ratios (SMRs), and **proportionate mortality ratio (PMR) studies were considered for inclusion in the meta-analysis.** Although studies reporting PMRs are often not relied upon or given much weight in critical reviews and syntheses due to their methodological limitations, **we included these studies** because previous reviews and meta-analyses have included them. Since estimated standard errors (SEs) of the REs were needed to calculate weights, only studies that reported confidence intervals (CIs) or other data that allowed for the calculation of SEs were included (see below). Studies that reported no exposed cases were excluded from the meta-analysis.

We can find no probative basis for NTP's dismissal of the findings of this most recent and rigorous meta-analysis.

Cancer at Other Tissue Sites

[No comment.]

III. Cancer Studies in Experimental Animals

[No further comment. *See* FCI 2/10 Comments at 9-11, 18-19, and 21.]

IV. Other Relevant Data

The Profile attempts to explain how exogenous inhaled formaldehyde, which does not enter the blood [17] to change normal endogenous concentrations,[18] can nevertheless travel to distant sites

[16] Profile at 6.

[17] *See* Lu *et al.* (2010).

to initiate the leukemogenic process. The Profile makes the following series of statements, which together we refer to as the "methanediol hypothesis":

> Although formaldehyde is a gas at room temperature, it hydrates rapidly and is in equilibrium with its hydrated form, methanediol (Fox et al. 1985); at room and body temperatures, the dominant form is methanediol.[19]
>
> However, there is some evidence for systemic distribution of formaldehyde in humans.[20]
>
> The mechanism by which formaldehyde causes toxicity at distal sites is unknown. The formation of methanediol (discussed above) helps to explain how a reactive chemical can be distributed and undergo metabolism throughout the body (Fox et al. 1985, Matubayasi et al. 2007). In addition, formaldehyde reacts reversibly with a variety of endogenous molecules, including glutathione, amino acids, and folic acid (Heck et al. 1982). These reversible products may be transported from the portal of entry to reach remote sites where free formaldehyde can then be released.[21]

The Profile tries to create a biologically feasible mechanism for leukemia induction by incorrectly claiming that exogenous formaldehyde enters the blood and causes adverse effects at sites distal to the nasal epithelium as a consequence of the formaldehyde-methanediol equilibrium (which greatly favors methanediol by a factor of approximately 7000) being disrupted, resulting in the release of free formaldehyde. However, the references cited in support of the "methanediol hypothesis, "i.e., Fox *et al.* (1985) and Matubayasi *et al*. (2007), are not relevant to the behavior of formaldehyde in living biological systems. Matubayasi *et al*. (2007) discusses the equilibrium of formaldehyde in very hot water (i.e., >200 ^{o}C, and Fox *et al*. (1985) provides a historical description of the use of formaldehyde in tissue fixation for histopathology.

In Fox *et al*. (1985), the authors observe that *"[t]he equilibrium between methylene glycol and formaldehyde in aqueous solution lies so far in favor of methylene glycol that the conversion of methylene glycol to formaldehyde by removal of formaldehyde can be used as a "real-time" clock, measurable in hours."* Much of the article involves discussion about the chemical fixation properties of a *10% formalin or 4% formaldehyde solution* that "*[i]s 1.3 molar by definition*." It is scientifically unwarranted to hypothesize about the biological activity of formaldehyde based on extrapolating from tissue fixing concentrations of 4% (i.e., 40,000 parts per million (ppm)) to normal endogenous concentrations of 2-3 ppm, which are at least 10,000 times less. *See* FCI 2/10 Comments at 11-12.

It also is noteworthy that formaldehyde as methanediol is already present in the blood and, in fact, has already penetrated every tissue in the body (i.e., distant sites) due to its ubiquitous presence. The equilibrium strongly favoring methanediol in biological systems, and not free formaldehyde, is essentially confirmed by Matubayasi *et al*. (2007), who concluded:

> *The formation of methanediol through the hydration reaction of formaldehyde is unfavored in the absence of solvent, and is favored by the solvent water more at*

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[18] *See* Heck and Casanova (2004), Casanova *et al.* (1988), Heck *et al*. (1985).

[19] Profile at 7.

[20] *Id*. at 9.

[21] *Id*. at 10.

> *a lower temperature. In the equilibrium between (unhydrated) formaldehyde and methanediol, the dominant form is methanediol at ambient conditions and the unhydrated form is found to be predominant in hot water above ~ 200° C.*

The extensive data on methanol provides indirect, but relevant additional data suggesting that inhaled formaldehyde does not lead to increased blood or tissue levels of formaldehyde. Inhaled methanol is readily absorbed into the blood, metabolized to formaldehyde and then to formate. Several tables in the Draft IRIS (2010) document on methanol illustrate normal background blood levels of both methanol and formate in humans, monkeys and rats, as well as levels following inhalation exposure to varying concentrations of methanol. Noteworthy is that even though blood methanol concentrations are clearly elevated following exposure to graded doses of methanol, formate levels remain constant. For example, as shown in one table (Table 3-3), exposure of monkeys to methanol at 0, 200, 600, and 1,800 ppm for 2.5 hr/day, 7days/wk for 348 days showed blood methanol concentrations of 2.4, 5, 11 and 35 mg/L while formate levels remained constant (i.e., 8.7, 8.7, 8.7 and 10 mg/L).

Given the conversion of methanol to formaldehyde (t1/2.≈ 3 hrs) with formaldehyde conversion to formate (t1/2 ≈ 1 min) in both rodents and humans, the lack of increased blood formate levels following chronic exposure to methanol suggests that formaldehyde levels are also not changed from normal endogenous levels following high dose exposure to methanol. No empirical data are presented in the IRIS document that supports a different conclusion. Thus, the data on methanol, an unequivocal "carrier" of formaldehyde into the blood, challenge the notion that inhaled formaldehyde, which doesn't even get into the blood to change normal endogenous concentrations, would somehow be transported to distant sites as a consequence of any hypothesized mechanism.

If the convoluted logic of the methanediol hypothesis is the only evidence that NTP can offer to explain the distant site (e.g., bone marrow) toxicity that is required to support any hypothesized mechanism for formaldehyde-induced leukemia, then NTP should not include such a discussion or offer such a conclusion in the final profile for formaldehyde. This is particularly the case since,

- Inhaled formaldehyde, even up to 15 ppm for 90 days, has no adverse effects on red or white blood cell counts or on the bone marrow of rats. M. Andersen (personal communication). These findings following 90 days exposure to 15 ppm of formaldehyde demonstrate that the initial events (i.e., myelotoxicity-driven pancytopenia) required for the development of leukemia do not occur.
- Distant-site toxicity was investigated by Lu *et al.* (2010) where rats were exposed via inhalation to ^{13}C-formaldehyde at 10 ppm for 1 and 5 days. While formaldehyde-DNA adducts from both endogenous and exogenous formaldehyde were readily detected in nasal epithelium after 1 or 5 days, no formaldehyde-DNA adducts from exogenous formaldehyde were detected at any site distal to the nose, including blood and bone marrow. This confirms prior work demonstrating that exogenous formaldehyde does not get past the nasal epithelium, and calls into question the findings reported by Zhang *et al.* (2010) involving formaldehyde-induced changes.
- The vast majority of more credible data show essentially no reported adverse hematological effects in humans or animals following either oral or inhalation exposure to formaldehyde.

V. Studies on Mechanisms of Carcinogenesis

A. Nasal Cancer

The Profile should be amended to reflect the recent *in vivo* study, Meng *et al.* (2010), suggesting that formaldehyde-induced mutagenicity is unlikely to play a role in tumorigenesis. Currently, the Profile states, "*Mutations in the p53 tumor-suppressor gene (at G:C base pairs) were found in formaldehyde-induced nasal squamous-cell carcinomas in rats, and all of the identified codon mutations have also been found in human cancers* (Recio *et al.* 1992)."[22] By this statement, NTP suggests that *p53* mutations are involved in the development of nasal tumors in rats subsequent to formaldehyde exposure. While various *in vitro* studies indicate that formaldehyde is mutagenic in a number of test systems,[23] Meng *et al.* (2010) demonstrates that formaldehyde-induced mutagenicity is unlikely to play a role in nasal tumorigenesis.

In Meng *et al.* (2010), F-344 rats were exposed to formaldehyde at concentrations of 0, 0.7, 2, 6, 10 or 15 ppm for 13 weeks with nasal epithelial tissues examined for the presence of one of the *p53* mutations that had been detected in the squamous-cell carcinomas induced by chronic formaldehyde exposure in Recio *et al.'s* (1992) two-year bioassay. In addition, because regenerative cell proliferation is considered a key event in formaldehyde-induced carcinogenesis,[24] nasal mucosal cell proliferation was monitored by bromodeoxyuridine (BrdU) incorporation. While a low spontaneous background level of *p53* mutation was detected, this level was not increased by formaldehyde exposure, even at tumorigenic doses. However, when measured by BrdU labeling, the percentage of proliferating cells increased with formaldehyde dose and was significantly increased at 10 and 15 ppm compared to controls.

These data, showing no increase in *p53* mutation but significant changes in regenerative cell proliferation following 13 weeks of formaldehyde exposure at tumorigenic doses, suggest that *p53* mutation is a late event that is not involved in the carcinogenic mode of action (MOA) in formaldehyde-induced carcinogenesis and that occurs only after other key events (*e.g.*, DNA-protein crosslinks, cytotoxicity, cell proliferation) have occurred.[25] The Profile should be amended accordingly. In addition, given the findings of Meng *et al*. (2010), the DNA-protein crosslinks in lymphocytes that were significantly associated with increased risk of higher serum *p53* levels, as reported by Shaham *et al.* (2003), are of questionable relevance to any conclusion suggesting that effects detected in lymphocytes (even if real) would have any relationship to a subsequent event such as the development of nasal cancer.

B. Myeloid Leukemia

The Profile does not explain sufficiently how formaldehyde can cause myeloid leukemia at distal sites and overlooks studies demonstrating the implausibility of this hypothesis. NTP begins its analysis by stating that *"[t]he endogenous concentration[of formaldehyde] in the blood of humans, monkeys, and rats is about 2 to 3 µg/g, and the concentration does not increase after inhalation of formaldehyde from exogenous sources*."[26] This statement must be reconciled with

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[22] Profile at 8.

[23] *See* ATSDR (1999); IARC (2006).

[24] *See* McGregor *et al.* (2006).

[25] *See* Meng *et al*. (2010).

[26] Profile at 9.

NTP's later claim that*, "[t]here is some evidence for systemic distribution of formaldehyde in humans.*"[27] The critical question, left unanswered, is how systemic distribution of additional formaldehyde occurs in humans (or any other species) with the unfounded implication that free formaldehyde is released when it is already distributed everywhere, bathing every tissue in the body, and even following exposure "*[formaldehyde] concentration does not increase.*" This convoluted logic should either be explained or eliminated from the document since it has no basis in reality.

NTP devotes a single sentence to a recent study by Lu *et al.* (2010) and otherwise appears to either intentionally ignore or not understand the implications of this study, which challenge NTP's assertion that systemic distribution of formaldehyde in humans is plausible. NTP states: "*Moreover, N2-hydroxymethyl-dG–DNA adducts have not been detected at distal sites in rats (such as the bone marrow, white blood cells, lung, spleen, liver, or thymus) (Lu et al.* (*2010*)."[28]

In Lu *et al.* (2010), male F344 rats were exposed to 10 ppm of ^{13}C-formaldehyde for one or five days (6h/day). Because of the 13C labeling it was possible to distinguish whether DNA adducts were formed from endogenous or exogenous formaldehyde. Following the 1 or 5 day exposures, blood was collected for lymphocyte isolation as well as tissue samples from nasal respiratory epithelium, spleen, thymus, lung and liver; bone marrow was collected from both femurs. DNA adducts from all tissues were subsequently prepared for analysis with the intentional bias of using five times more DNA from distant site tissues to enhance the ability to detect formaldehyde-DNA adducts if they existed.

While formaldehyde-DNA adducts from both endogenous (^{12}C) and exogenous (^{13}C) formaldehyde were detected in nasal epithelium after either 1 or 5 days of exposure, no ^{13}C-formaldehyde-DNA adducts were detected in any tissue distal to the nasal epithelium, including the lung, spleen, liver, thymus, bone marrow or lymphocytes. As described by the authors, "*The absence of exogenous formaldehyde-induced DNA adducts and crosslinks in other tissues supports the conclusion that genotoxic effects of inhaled formaldehyde are implausible at sites remote to the portal-of-entry.*" Additionally, with respect to the methanediol hypothesis discussed above (i.e., methanediol explains the transport of inhaled formaldehyde to distant sites), the authors explicitly addressed this issue noting, "*Furthermore, by monitoring the transitions that would occur if there was any hydrogen-deuterium exchange, we have demonstrated that neither inhaled formaldehyde, nor methanediol derived from inhaled formaldehyde reaches sites distant to the portal of entry.*" The inability to detect ^{13}C-formaldehyde-DNA adducts in the blood or bone marrow strongly diminishes the likelihood of formaldehyde-induced leukemia as a consequence of distant site toxicity, and the Profile must discuss this implication. *See* Feb. 11, 2010 Comments at 13.

Nevertheless, NTP asserts that "*Numerous studies in humans and experimental animals have demonstrated that inhaled formaldehyde can cause toxicity, genotoxicity, and cancer at distal sites.*"[29] The draft Profile discussion (Cancer Studies in Experimental Animals at pp. 6-7) does not support this statement, particularly if the studies by Soffritti et al. (1989, 2002), which have been dismissed by both the Agency for Toxic Substances and Disease Registry and the U.S.

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[27] *Id*.

[28] *Id*.

[29] Profile at 9.

Food and Drug Administration as unreliable, are eliminated from consideration. See Feb. 11 2010, FCI Comments at 10.

The draft Profile did not properly characterization the data concerning the hematological toxicity of formaldehyde in humans. The Profile states:

> *Regardless of the proposed mechanism, hematological toxicity of formaldehyde would be expected, and adverse hematological effects have been reported in some, but not all, studies in humans. However, no adverse hematological effects have been reported in subchronic or chronic studies in experimental animals (Dean et al. 1984, Appelman et al. 1988, Kamata et al. 1997*).[30]

There is little, if any, credible evidence of formaldehyde-induced hematotoxicity in humans, which is consistent with the above described lack of hematotoxicity in animal studies. *See* FCI 2/10 Comments at 14-15. While an accidental ingestion of a large quantity of formaldehyde was reported to cause an intravascular coagulopathy,[31] several reports of human ingestion of lower doses have not shown any effects on the blood or blood-forming organs.[32]

NTP appears to rely on a review of literature without critically analyzing the underlying studies. The Profile states:

> A review of the Chinese literature reported *that decreased white blood cell counts were observed in most studies of formaldehyde-exposed workers; in the largest study, exposed workers had higher percentages of blood abnormalities (decreased white blood cell and platelet counts and abnormal hemoglobin levels) (Tang et al. 2009).*[33]

However, the data reviewed and reported by Tang *et al.* (2009) do not provide a credible basis for assuming that formaldehyde exposure is a cause of adverse hematological effects. The only study cited in Table 9 of Tang et al. that was in English was by Kuo *et al.* (1997) conducted on 50 hemodialysis nurses and controls from four hospitals in Taiwan which concluded that the white blood cell counts were significantly lower in the exposed group compared to controls. However, this study is not credible because the formaldehyde analytical data are suspect and the overall formaldehyde levels were implausibly low (*e.g.*, mean personal sampling concentrations of 0.015, 0.017, 0.033, and 0.054 ppm and area sampling concentrations of 0.231, 0.022, 0.219, 0.006 and 0.237 ppm) in the four hospitals studied. *See* Feb. 11, 2010, FCI Comments at 15. If the other, un-translated studies are similar, either with respect to the hematology results or the exposure concentrations, then there is little basis for assuming they are reporting a formaldehyde-related effect. This may be important if, as reported by Kuo et al. (1997) that the reported formaldehyde concentrations are similar to the exposure levels of controls reported in Zhang *et al.* (2010).

NTP proceeds to discuss Zhang *et al.* (2010), and states:

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[30] *Id*. at 10.

[31] Burkhart *et al.* (1990).

[32] *See* Eells *et al.* (1981); Freestone and Bentley (1989); Koppel *et al.* (1990).

[33] *Id*. at 11.

> *Zhang et al. (2010) found that formaldehyde-exposed workers had lower counts of white blood cells, granulocytes, platelets, red blood cells, and lymphocytes than nonexposed workers, and that a subset of workers showed an increased frequency of aneuploidy of chromosomes 7 (monosomy) and 8 (trisomy). Monosomy 7 and trisomy 8 are associated with myeloid leukemia (Johnson and Cotter 1997, Paulsson and Johansson 2007). In addition, formaldehyde exposure in vitro caused a decrease in colony-forming progenitor cells (erythroid burst-forming units, erythroid colony-forming units, and granulocyte, erythrocyte, monocyte, and megakaryocyte colony-forming units).*[34]

Given the central importance of this study to the issue of whether exposure to formaldehyde might be implicated in the etiology of myeloid leukemia, it is not clear why these preliminary results are accepted without reservation. Since this study played a pivotal role in the conclusions reached by IARC (2009) concerning an association between formaldehyde exposure and leukemia, it is surprising that the NTP did not address its strengths and weaknesses. For example, since the hematology data are pooled, and all values for the exposed workers are well within the normal range for every parameter examined, is there any possibility that the reported results are not due to formaldehyde, but rather to the equally possible likelihood that several individuals in this group had colds or other infections that influenced their individual blood counts? It is also important to note that chromosomes 7 and 8 are minimally relevant to leukemia, and their count number in peripheral blood lymphocytes is not known to have *any* predictive value for future disease development. Chromosomes 7 and 8 are not usually involved in leukemia as shown by the fact that in 122 acute myeloid leukemia (AML) patients in China, none had monosomy 7 and only four had trisomy 8.[35] Moreover, there is no existing accepted diagnostic test in clinical medicine, hematology or hematopathology that can establish the presence of leukemia, or increased risk of developing leukemia, by detection of monosomy 7 or trisomy 8 in cultured myeloid progenitor cells from peripheral blood. Nevertheless, Zhang *et al*. (2010) equated formaldehyde with benzene and implied that their results were predictive of future leukemia. It is also unknown why Zhang et al. (2010) examined only chromosomes 7 and 8 while ignoring all other chromosomes, translocations and the common genetic lesions associated with leukemia. Does NTP have any opinions on these issues and importantly does it agree with the conclusion of Zhang et al. (2010) that formaldehyde acts similarly to benzene in initiating leukemogenesis?

Finally, it should also be noted that while the International Agency for Research on Cancer (IARC 2009) concluded that formaldehyde exposure was associated with leukemia, its skepticism about this association (based on an inability to identify a plausible mode of action), as stated in a previous evaluation (IARC 2006), still remains. For example, as discussed by IARC (2006), the hypothesis that formaldehyde may cause leukemia "…*raises a number of mechanistic questions, including* ***the processes by which inhaled formaldehyde may reach a myeloid progenitor****."* IARC continues, "[*a*] *clastogenic product of FA could conceivably be formed in the blood and circulate to the bone marrow although* ***this has not been suggested in the literature.***" And finally, "*…it is possible that circulating myeloid progenitor stem cells could be the source of leukemia....such cells are present in the blood and plausibly could be exposed*

[34] Id. at 10.

[35] *See* Zheng, *et al.* Cytometry Part B: Clinical Cytometry, 74B, pp 25-29 (2007).

to formaldehyde in the respiratory tract vasculature; however, ***there is no known prototype for such a mechanism of leukemogenesis****."* [emphasis added]

Despite the results of the Zhang *et al.* (2010) study, it appears that none of the questions raised by IARC can be answered even today. Consequently, this leaves unsettled the conundrum of how inhaled formaldehyde, which does not enter the blood to change the endogenous concentrations normally present, which is not transported to distant sites as methanediol with release of free formaldehyde, which cannot be detected (as ^{13}C-FA-DNA adducts) in white blood cells or bone marrow following inhalation of 10 ppm, and which has never induced leukemia in an animal study, is capable of causing leukemia in humans, not to mention myeloid leukemia, specifically.

In the **Myeloid Leukemia** discussion (p. 9), after essentially dismissing the important implications of Lu et al. (2010) which clearly demonstrated that inhaled exogenous formaldehyde does not go beyond the nasal epithelial tissues (including to white blood cells and bone marrow) it is nevertheless stated that, "…*there is some evidence for systemic distribution of formaldehyde in humans."*

The first "evidence" cited for the above statement is a study by Pala et al. (2008) in which implausibly low levels of formaldehyde (i.e., 75th %ile of 26 ug/m^3 or 21 ppb) were associated with increased formaldehyde serum binding to human serum albumin (FA-HSB). Importantly, because there was no significant trend between formaldehyde air concentrations and FA-HSB and since smoking (which contains formaldehyde) was not controlled the reported results have little, if any meaning, with respect to providing evidence for systemic distribution of formaldehyde. Inexplicitly not mentioned, however, are the more relevant results from this study showing that formaldehyde exposure was not associated with increased frequencies of the effect markers examined (i.e., CA, MN and SCE) thereby showing that genetic damage from exogenous formaldehyde did not occur.

The draft Profile appears to emphasize positive studies (occasionally indicating that negative studies also exist), suggesting an assumption that a positive study (whatever its shortcomings) is controlling. While negative studies need to be carefully reviewed to determine whether the study design and/or the experimental conditions were appropriate to detect an effect, the same criteria (i.e., quality and plausibility) also need to be applied to positive studies. With respect to demonstrating a potential systemic genotoxic/mutagenic effects from formaldehyde there are only two possible sources of data: animal experiments and human biomonitoring studies.

For example, in an animal inhalation study with formaldehyde (Speit et al., 2009) no systemic genotoxic effects were observed under the experimental conditions used, i.e., 4 weeks inhalation up to 15 ppm. This study is reliable because it was performed under GLP conditions in accordance with international guidelines and supports the negative results of the inhalation study by Kligerman et al. (1984) and even a negative study after i.p. injection of formaldehyde (Natarajan et al., 1983). Notably, the Speit et al. (2009) study is not even mentioned in the draft substance profile.

However, there are many positive studies and it is not always possible to identify fundamental problems or shortcomings, but when present it is critical that they be addressed. For example, the frequently cited study by Kitaeva et al. (1990) is not reliable and substantial uncertainties exist with regard to the number of animals used per data point, the number of cells actually evaluated per animal, the time point of the analysis with the high frequency of hypoploidies

indicating problems with the preparation of metaphases. If those performing reviews of the data are not qualified to critically review each study (positive or negative) this diminishes the credibly of any analyses, particularly if it is purported to represent the weight of evidence. This is aptly illustrated (p. 10) where it is stated that, "*Inhaled formaldehyde also caused DNA single-strand breaks in the liver and lymphocytes of male rats (Im et al. 2006), dominant lethal mutations in rats (Kitaeva et al. 1990), and heritable mutations in mice (Liu et al. 2009); however,* ***most studies found no cytogenetic effects*** *(NTP 2010)*' (emphasis added). The draft Profile does not take into account (or even cite) the detailed critique by Speit (2006) concerning the biological plausibility of the results reported Im et al. (2006). And finally, with respect to the overarching issue of basing decisions on the weight of the evidence, how can the uncritical use of selected data to make a point (e.g., formaldehyde has genotoxic effects distal to the nasal epithelium) be reconciled with the last part of the above statement that *most studies found no cytogenetic effects (NTP 2010)*? This suggests that formaldehyde does not have systemic genotoxic effects undermining the position that any of these highly selected (and non representative) data are relevant to the tortured argument used to support the biological plausibility of formaldehyde-induced leukemia.

With respect to the large number of human biomonitoring studies, in most cases, it is difficult to assess the quality of many of them (e.g., whether samples from exposed subjects and controls were collected and processed concurrently, whether slides were coded and analyzed in a random order, etc.). For example, in an *in vitro* study with human blood (Schmid and Speit, 2007) it was clearly demonstrated shown that the induction of genetic endpoints in these tests required high levels of persistent damage and that the required conditions simply cannot be met by any environmental or workplace exposure. Also characterized was the differential sensitivity of the typical endpoints measured (i.e., comet, SCE, micronucleus). This is in contrast to the study by Costa et al. (2008) in which it was reported that FA-induced genetic effects measured by the same tests all had the same sensitivity, a highly unlikely outcome. Finally, while there are reports on formaldehyde-induced DNA strand breaks (that can be due to repair activity or are artifacts) this could not be confirmed in V79 cells. (Speit et al., 2007; also not cited in the substance profile). Importantly, and directly relevant to the quality, rigor, and transparency of the review in the NTP formaldehyde substance profile is that with one exception (i.e., Speit et al. 2000), none of the other directly relevant studies by Speit and colleagues are cited or discussed. Is this because these carefully conducted studies produced results that are not supportive of the overall theme of this review that formaldehyde is capable of causing myeloid leukemia? If there are other reasons, these should be explicitly stated.

Numerous studies have investigated the potential *in vivo* genotoxicity (i.e., DPX, SCE, MN or CA) in the peripheral lymphocytes of occupationally exposed workers compared to unexposed controls (Bauchinger and Schmid, 1985; He et al., 1998; Yager et al., 1986; Ying et al., 1997, 1999; Vasudeva and Anand, 1996; Thomson et al., 1984). These studies led to inconsistent and conflicting results and a critical assessment of the majority of these studies (positive or negative) is difficult because of shortcomings in the study design and/or the evaluation of the data.

On the basis of comprehensive *ex vivo* experiments with cultures of human lymphocytes it is highly uncertain whether *in vivo* exposure to formaldehyde can actually lead to positive effects in genotoxicity tests with lymphocytes of exposed subjects. SCE and MN measured in blood cultures of exposed humans are formed *ex vivo* during the proliferation of lymphocytes. The

formation of these cytogenetic effects as a consequence of *in vivo* exposure requires that the cells sampled retain the increased levels of DNA damage. This damage has to persist up to replication and cell division. It is known that lymphocytes start replication about 24 hours after stimulation. Due to the rapid repair of DPX the probability that DPX will persist and effects will occur is remote. Furthermore, it is highly improbable that DPX would accumulate in lymphocytes after inhalation exposure in sufficient amounts since the conditions necessary to induce measurable effects (i.e., high DPX levels and persistence of DPX until S-phase) are simply not achieved (Schmid and Speit, 2007). Most likely, the positive effects reported are chance findings or due to other kind of exposures of the populations studied. Therefore, human biomonitoring studies should be interpreted with great caution as a supporting argument for systemic genotoxic/mutagenic effects of formaldehyde. Most importantly these markers are for circulating lymphocytes, and it has never been demonstrated that these effects occur in stem cells or HPC that can then somehow transition to leukemia. With respect to this latter issue, there is no evidence cited by Zhang et al. (2009) that any of the proposed events actually occur other than that *"…**one can imagine** the targeting of sufficient stem cells through these two alternative models to induce leukemia…"* [emphasis added] This key issue should be based on data rather than on imagination. For example, the inability of formaldehyde to induce systemic genotoxic/mutagenic effects (i.e., damage bone marrow or circulating lymphocytes directly) has recently been demonstrated in comprehensive *in vivo* animal experiments (Speit et al. 2009). Inhalation of formaldehyde in a 28-day study at concentrations up to 15 ppm did not produce any effects in the comet assay (DNA strand breaks and DPX), the SCE test or the MN test with peripheral blood. One can only imagine why these results were not considered in the draft substance profile or why the Speit et al. (2009) study was not cited.

Finally, the overarching question that needs to be addressed, and is not, is why should we expect (or speculate) that formaldehyde is systemically available and can induce systemic genotoxic effects in white blood cells or other target organs despite the abundance of experimental evidence demonstrating that formaldehyde is not delivered to distant sites. Recent data (Neuss et al., 2010) clearly show that formaldehyde can act on cells of first contact but is not released from these cells and able to damage other nearby cells. In this *in vitro* co-cultivation study with primary human nasal epithelial cells (HNEC) and isolated lymphocytes possible effects on lymphocytes (i.e., DPX) was investigated to determine whether reactive FA can be passed on from nasal epithelial cells (site of first contact) to lymphocytes located in close proximity and induce DNA damage in these cells. The results clearly showed that co-cultivation of lymphocytes with HNEC exposed to formaldehyde for 1 h causes a concentration-related induction of DPX in lymphocytes when co-cultivation takes place in the exposure medium. However, when the exposure medium was changed after FA treatment of HNEC and before lymphocytes are added, no induction of DPX could be measured in lymphocytes even after high FA concentrations (300 muM) and extended co-cultivation (4 h). These results suggest that formaldehyde that enters nasal epithelial cells is not released and does not damage other cells in close proximity to the epithelial cells. These results do not support the proposed hypothetical mechanism for FA-induced leukemia (P. 10) by either damaging circulating hematopoietic stem cells or hematopoietic progenitor cells in nasal passages, which then travel to the bone marrow and become initiated leukaemic stem cells. Notably, the Neuss et al. (2010) study was not cited in the NTP draft substance profile.

VI. Properties

[No comment.]

VII. Use

[No comment.]

VIII. Production

[No comment.]

IX. Exposure

A. Environmental Exposure

[No comment.]

B. Occupational Exposure

[No comment.]

X. Conclusion

The above review of the key scientific data does not support a finding that there is sufficient evidence to classify formaldehyde as a *known human carcinogen*. In many critical instances, there is no statistically significant data to support such a characterization or the interpretation in the draft Profile does not address or adequate explain its determinations in light of study data to the contrary. For these reasons, we ask that the Board not concur with the conclusion of the draft Substance Profile listing of formaldehyde as *known to be a human carcinogen* in the 12th RoC.

If you have any questions or seek additional information from FCI and its science consultants, please do not hesitate to contact me at 703-875-0710 or bnatz@formaldehyde.org.

Sincerely,

[Redacted]

Betsy Natz
Executive Director
Formaldehyde Council, Inc.

Enclosure

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